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Studies of an Analogous Enzyme of Thiamin Phosphate Synthase

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The biosynthesis of thiamin involves the separate formation of two compounds: a thiazole and a pirimidine which are linked to form thiamin phosphate by the Thiamin Phosphate Synthase (TPS) enzyme. Twelve genes are involved in thiamin biosynthesis in prokaryotes, six are required for the thiazole biosynthesis (*ThiFSGHI,* and *dxs*), one is involved in the pyrimidine biosynthesis (*ThiC*), four code to kinases (*ThiD, ThiM, ThiL, and pdxk*) and one is required for the linking of the thiazole and pyrimidine (*ThiE*).(1)

Several organisms whose genomes have been wholly sequenced, in spite of being autotrophs for thiamin, lack one or more homologs to the *Escherichia coli* genes for the thiamin biosynthesis pathway. *Thermotoga maritima*, a thermophilic eubacteria, lacks the *thiE* gen that codes for the TPS. In a bioinformatic search, with a negative correlation strategy, the gene TM0790 was identified as candidate to carry out the function of *thiE* in *T. maritima*. That function was confirmed by complementation assays of an *Escherichia coli* strand deleted of the thiE gene. TM0790 is a hybrid gen that has in its 5' region an homologous to the *thiD* gen of the same pathway, and in its 3' region a gene which only hs homologous in archaeas and this is the region that by itself can complement the *thiE* function (2). The phenomena of lacking genes is not exclusive for the thiamin biosynthesis pathway, it is also seen in the route of synthesis for many vitamins, for example, biotin and pyridoxal.

The TPS from *Bacillus subtilis* is an alpha/beta protein with a Triosephosphate isomerase fold (3) but nothing is known about the fold for the protein from *T. maritima* that complement the function of TPS. The aim of this project is to determine the structure of the enzyme codified by the hybrid gen TM0790 from *T. maritima*, to compare structural and functionally with its analogous TPS from *Bacillus subtilis*, and to continue the search of analogous enzymes in the biosynthesis pathway of biotin, pyridoxal, and other vitamins.

In the visit done between July 17th to 22nd, we did diffraction and cryoprotection measures for several crystals that we obtained in a first screening. These crystals diffracted as salt. Now, in another crystal growing conditions, we have obtained protein crystals which diffraction at 4 A resolution and we are exploring similar conditions to have crystals with a high quality and resolution diffraction pattern.

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References:

1. Begley T. Downs, D. Ealick S. McLafferty F. Van Loon A. Taylor S. Campobasso N. Chiu H. Kinsland C. Reddick J. Xi J. 1999. Thiamin Biosynthesis in Prokarvotes, Arch. Mocrobiol. 171: 293-300.
2. Morett E. Korbel J. Rajan E. Saab-Rincon G. Olvera L. Olvera M. Schmidt S. Snel B. Bork P. 2002. "Predictin and Confirmation of Extensive Protein Displacement in Thiamin Biosynthesis. Submitted article.
3. Chiu H. Reddick J. Begly T. Ealick S. 1999. Crystal Structure of Thiamin Phosphate Synthase from Bacillus subtilis at 1.25 A Resolution. Biochemistry 38: 6460-6470.